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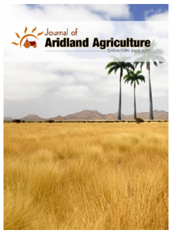
Research Article



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Evaluation of drought resilience of two Kurdish rice genotypes induced by polyethylene glycol (PEG-8000) at the early growth stage

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ABSTRACT

Drought is being regarded as a serious threat to the growth and production of sensitive crops like rice. Selecting of tolerant genotypes and pre- sawing treatments were aimed in the study. To find out the efficiency of seed priming to mitigate drought stress effects of two rice genotypes; Se-mahee (G1) and Shesh-mahee (G2) and their resilience capacity. Three primming methods: hydropriming (P0), osmo-priming with 50 g/L PEG-8000 (P1), and osmo-priming with 100 g/L PEG-8000 (P2) later on germinated under drought stress simulated with two osmotic potentials, -0.79 (D1) and -1.58 bars (D2). The highest germination percentage (G%) was recorded due hydro and 50 g/L PEG-8000 under control condition. The growth characters of seedlings; plumule and radicle lengths and their dry weights, as well as their vigor were significantly improved under moderate drought stress due to their previous hydropriming and 5% PEG. Thus, hydropriming and osmo-priming regarded as the best preconditions of seeds to stimulate drought tolerance. Se-mahee had superior resilience in the mean of having higher seedling vigor and root: shoot ratios under drought stress. The findings of the study could underscore the crucial role of seed priming for stress management strategy for enhancing crop resilience under water shortage conditions. As well as the reduced performance of Shesh-mahee under severe drought (-1.58 bars) compared to Se-mahee could suggest the higher resilience and adaptation of the genotype under drought stress conditions.

KEYWORDS: Resilience, PEG, Rice, Seed vigor, RWC, Proline

INTRODUCTION

Rice (*Oryza sativa* L.) is the second staple food after wheat consumed by more than half of the world's human population (Sagar *et al.*, 2020). Globally climate change is the main cause for agriculture loss mainly abiotic stress factors (Oladosu *etal.*, 2019). Sensitive plants like rice cannot survive under water shortage conditions, because it has a negative impact on critical stages of rice crop growth and development. Nearly 50% of rice production in the world is impacted by drought stress (Siddique *et al.*, 2023). Water scarcity affects negatively on cell division, stomatal conductance which controls transpiration and photosynthesis rate (Tuna *et al.*, 2010). The impact of water stress was greater during the germination and seedling stage, and eventually on plant yield (Tsago *et al.*, 2014). Water absorption and seed imbibition is the first step for starting germination and its rate greatly depends on the chemical nature of the seed like; protein, mucilage and pectin, they have strong hydrophilic nature that can adsorb more water as compared to starch due their greater number of capillary spaces (Fathi & Tari, 2016). Water acts as a necessary medium for hydrolytic enzyme activation for breaking down the stored food in endosperm such as proteins, lipids, and carbohydrates, despite to its role in solubilizing the metabolites to be transported (Białecka & Kępczyński, 2010). For example, amylase enzyme is a hydrolytic enzyme that breaks the starch to simple sugars, to generate ATP (adenosine tri phosphate) the cell energy for radicle and plumule development (Zeid & Shedeed, 2006). In response to drought stress, plants could develop a defense mechanism by conserving water and provide it to the aerial parts (Fanti & de Andrade Perez, 2004). Agronomic practices like soil fertilization, irrigation techniques, bio stimulants and selecting tolerant varieties were regarded as a valuable strategy for reducing the harsh effects of abiotic stresses (Hossain *et al.*, 2021). Seed priming is one of these practices management to protect various crops against challenged environmental factors without a considerable effect on crop productivity (Bittencourt *et al.*, 2004). It is a pre-sowing treatment that leads to more efficient seed germination (Beckers & Conrath, 2007). Previous studies on seed priming insured better seed germination, seedling emergence and establishment, improved growth and productivity of rice (Jisha & Puthur, 2016; Samota *et al.*, 2017; Kavitha *et al.*, 2020). Zhang *et al.* (2015) and Marthandan *et al.* (2020) concluded that primed seeds generally improved the early emergence, uniform germination by reducing the imbibition time, pregerminated enzyme activation and increasing metabolite production (Hussain *et al.*, 2018). Priming the seeds in low water potential solutions before germination remains one of the interesting topics to be studied. Because low osmotic potential solutions control the water level needed to complete seed germination that could be sufficient for the activation of necessary metabolic and physiological processes in the plant cells to initiate the germination process (Ibrahim, 2016). Osmo priming is a common priming technique through using different chemicals like; $CaCl_2$, KNO_3, KCl, K_3PO_4, NaCl, PEG, and mannitol for improving seed germination and seedling establishment (Lei *et al.*, 2021). Among these polyethylene glycols (PEG) most commonly used as priming osmoticum can simulate drought stress at the early stage of growth (Zhu *et al.*, 2021). PEG is a high molecular weight, inert chemical, cannot pass through the cell wall because of its large size and induces water stress without causing any physiological damage to the seed embryo (Qadir, 2018). Plant response to the stress varies according to the plant species, genetic nature, and growth stage. The tolerance of a genotype is usually related to morphological and physiological adaptation to the drought condition (Ghebremariam *et al.*, 2013). The scenario of climate change, global warming and population nowadays could be regarded as one of the challenged factors to the water demand for crop growth. Thus, screening the resilient rice genotypes to climatic stresses especially drought might have a crucial role in distinguishing the tolerant local varieties as a base for further breeding programs. As well as, to explore the effectiveness of PEG as an osmoticum to induce drought stress and optimize its effect as a priming agent and suitable priming time for improving germination behavior and seedling establishment under water shortage condition to provide insights for future research.

MATERIALS AND METHODS

Rice Genotype Materials

Two Kurdish rice genotypes Se-mahee (G1) and Shesh-mahee (G2) were used in the study that cultivated locally in Akre town- Duhok province- Iraq. Rice seeds were washed with distilled water for three times. Soaked in 70% ethanol for thirty seconds and then kept in NaOCl solution (2%) for fifteen minutes. After sterilization process the seeds were washed four times with distilled water to remove any traces of the chemicals used for sterilization, finally they were left to dry at room temperature (Qadir *et al.*, 2016).

Induction of Drought Stress Using Poly Ethylene Glycol (PEG-8000)

Drought stress was simulated by exposing seeds to two osmotic potentials (D1 and D2), -0.79 and -1.58 bars created by dissolving 50 and 100 g PEG-8000 in sterilized distilled water to make 1 L solution. The control group (P0) was maintained by using sterilized distilled water alone. The osmotic potential of the two concentrations was calculated according to Michel and Kaufmann (1973).

$$\text{Osmotic Potential (OP)} = (-1.18\times10^{-2})\times C - (1.18\times10^{-4})\times C2 + (2.67\times10^{-4})\times C\times T + (8.39\times 10^{-7})\times C2T$$

Where, C=PEG concentration, T=Temperature (29 °C)

Germination Test

The sterilized rice seed genotypes were primed in sterilized distilled water (P0), 50 and 100 g/L PEG-8000 solutions for 24 hours as P1 and P2. Then ten seeds of each genotype according to the treatment combinations were placed in a 9 cm diameter Petri dish bedded with two sterilized Whatman No. 1 filter paper. The filters were kept moist by adding 9 mL of each concentration and the Petri dishes were sealed with parafilm to avoid moisture loss and incubated in a growth chamber at a constant temperature of 25±2 °C and moisture of 65%.

Studied Parameters

Seed germination indices

After the emergence of 1 mm radicle length the seed regarded as germinated seed and the number of germinated counted in each replicate of a treatment. The following indices were calculated: Germination percentage (G%) = germinated seed number/Total planted seeds number * 100 (ISTA, 1999). Promptness index (PI) PI = (nd1 × 1.0) + (nd2 × 0.75) + (nd3 × 0.50) + (nd4 × 0.25). Where, nd1, nd2, nd3 and nd4 are the germinated seeds after the 1st, 2nd, 3rd and 4th day, respectively (George, 1967). Germination stress tolerance index (GSTI) = (PI of stressed seeds/PI control seeds).

Growth characters of seedlings

After 12 days the following growth characters were obtained:

Plumule and radicle length in centimeter (PL and RL)

Shoot length was measured from the shoot base to the tip of the longest leaf and root length was measured from the root base to the root tip. Then radicle to plumule ratio (R: P)=radicle length/plumule length calculated.

Plumule and radicle dry weight in grams (PDW and RDW)

Roots and shoots were separated from the seedlings immediately after harvest and fresh weights were recorded. Then dry weight of each obtained by drying them in an oven 72 °C for 48 hours and weighed directly.

Physiological indices

Depending on the growth measurement values, the following physiological indices were calculated:

a. Seedling vigor index (SVI) = G% × (Root length + Shoot Length) (ISTA, 1999)
b. Plumule length stress index (SLSI) (%) = [plumule length stressed plant/plumule length of control plants] x 100 (ISTA, 1999)
c. Radicle length stress index (RLSI) (%) = [radicle length stressed plant/radicle length of control plants] x 100 (AOSA, 2024)
d. Root elongation speed (RES) = root length/day required for germination
e. Shoot elongation speed (PES) = shoot length/day required for germination (ISTA, 1999)

Relative water content (RWC)

Fresh weight and dry weight of the seedlings were measured previously to obtain the growth characters. The turgid weight was obtained by keeping the seedlings in distilled water for 24 hours and the seedlings were weighed to obtain the turgid weight. The relative water content (Smart & Bingham, 1974) was determined according to the following equation:

$$\text{Relative water content (RWC)} = \text{Fresh weight/Dry weight} - \text{Turgid weight-Dry weight}$$

Water saturation deficit

Water saturation deficit is calculated according to the formula below (Baque *et al.*, 2018).

Water saturation deficit (WSD) = 1- Relative water content

Proline content

0.05 g of shoot was extracted in 3 mL of 3% of 5-sulphosalicylic acid. The extract was then centrifuged at 2000 round for 15 min. The clear supernatant was carefully poured into a glass vial and 2 mL of 5-sulphosalicylic acid was added to the residue and centrifuged for a second time to ensure complete extraction. The second supernatant was mixed with the first one and thoroughly mixed. Two mL of the extract was mixed with 2 mL of 3% ninhydrin reagent and 2 mL of glacial acetic acid. The mixture was kept in a water bath (100 °C) for 1 hour and then cooled at room temperature and gradually in an ice bath. Finally, 4 mL of toluene was added and mixed for 15 seconds. The mixture separated into two layers. The upper part was taken and the absorbance of the mixture was measured at 520 nm in a spectrophotometer (Bates *et al.*, 1973). The proline content of the leaf was expressed in μg/g FW (fresh weight of shoot).

Experiment Design and Data Analysis

The study was laid out as a factorial experiment with complete randomization of the treatments to the experimental units. The combined effect of two local Kurdish rice genotypes (G1 and G2), three priming solutions (P0, P1 and P2), and three drought stress levels (D0, D1 and D2). Each treatment was replicated five times [2 genotypes (G) * 3 priming times (S) * 3 levels of drought (P) = 18 treatment combinations * 5 replications = 90 experimental units]. The data was analyzed using the statistical package for the social sciences (SPSS) version; 25. The analysis of variance (ANOVA) was calculated to find the general significant difference. Duncan's test was used to calculate the pair-wise comparison between each pair of means of the studied characters at a significance level of 1% for laboratory measurements.

RESULTS AND DISCUSSION

The data belong to the indices of seed germination; germination percent (G%), promptness index (PI) and germination stress tolerance index (GSTI) shown in Table 1. A significant variation was exhibited among the mean values of all studied indices. The mean values of G% were 96.67 and 95.67 scored the highest value at (G1P0D0 and G1P1D0), when the seeds were soaked in distilled water and 50 g/L PEG-8000 and no drought stress was induced by G1. The same treatments in addition to G1P1D1 were recorded the highest value of PI, means inducing the first level of drought was not affected significantly on the speed and uniformity of seed germination. As well as a high PI (14.33) was observed in Shesh-mahee genotype when primed in distilled water with no induce of drought stress (G2P0D0). The highest germination stress tolerance index was recorded when no drought stress was induced by the two genotypes (G1P0D0, G1P1D0 and G2P0D0). But Se-mahee was able to cope -0.79 bars osmotic potential by recording GSTI of 0.87 which is not significantly differ from the previous set (G1P0D0, G1P1D0 and G2P0D0), because of its former priming with 50 g/L PEG-8000 (G1P1D1). The lowest values of G% were 28.23 and 30.00 recorded by G2P0D2 and G2P2D2, it means that the priming solutions did not significantly affect on minimizing the drought stress (-1.58 bars) effect on the number of germinated seeds of Shesh-mahee genotype. The lowest values of PI (1.48, 2.00, and 1.50 and GSTI (0.23, 0.14, and 0.10) were recorded when G2 was subjected to -1.58 bars osmotic potential with the three priming types (G2P0D2, G2P1D2, and G2P2D2). It can be concluded that Se-mahee (G1) as compared to Shesh-mahee (G2) was superior in

Table 1: Effect of drought stress levels and priming types on germination indices of two Kurdish rice genotypes

	Germination percent (G%)	Promptness index (PI)	Germination stress tolerance index (GSTI)
G1P0D0	96.67±3.33^{a}	16.08±0.08^{a}	1.00±0.005^{a}
G1P0D1	77.34±2.32^{bcd}	7.83±0.04^{c}	0.48±0.031^{de}
G1P0D2	65.12±2.98^{def}	5.18±0.03^{d}	0.30±0.013^{ef}
G1P1D0	95.67±3.33^{a}	16.07±0.44^{a}	1.00±0.002^{a}
G1P1D1	86.67±3.21^{abc}	14.00±0.66^{a}	0.89±0.041^{a}
G1P1D2	76.67±2.54^{bcd}	10.08±0.79^{bc}	0.63±0.053^{cd}
G1P2D0	93.33±5.12^{ab}	9.25±0.72^{bc}	0.58±0.04^{d}
G1P2D1	80.00±5.78^{abcd}	7.92±0.65^{c}	0.49±0.042^{de}
G1P2D2	66.67±2.34^{def}	5.50±0.72^{d}	0.34±0.044^{ef}
G2P0D0	73.33±4.23^{cd}	14.33±0.46^{a}	1.00±0.003^{a}
G2P0D1	48.33±1.65^{fgh}	3.51±0.21^{de}	0.35±0.32^{ed}
G2P0D2	28.23±2.13^{i}	1.48±0.18^{e}	0.23±0.011^{g}
G2P1D0	70.00±5.77^{cde}	10.92±0.55^{b}	0.76±0.038^{bc}
G2P1D1	43.33±3.35^{ghi}	4.33±0.30^{d}	0.30±0.021^{f}
G2P1D2	33.33±2.99^{hi}	2.00±0.14^{e}	0.14±0.011^{g}
G2P2D0	53.33±2.98^{efg}	5.12±0.67^{d}	0.36±0.046^{ed}
G2P2D1	50.00±5.77^{fgh}	3.67±0.74^{de}	0.26±0.051^{fg}
G2P2D2	30.00±5.77^{i}	1.50±0.43^{e}	0.10±0.031^{g}

its tolerance ability for drought stress and its response for priming for the studied indices. It can be concluded that hydropriming and osmo priming of seeds worked as a hardening process for the seeds before drought inducement. Due to priming the moisture content and oxygen will be available that induce the activation of hydrolytic enzymes; amylases, cellulases, and xylanases to convert carbohydrates, proteins and lipids into simpler compounds for generating the cellular energy (ATP) for pre-germination metabolism (Zulueta-Rodríguez *et al.*, 2015). Hydropriming improve the seedling vigor, uniform germination, early seedling emergence, crop growth, and development in chickpea seeds sweet basil (Kalhori *et al.*, 2018), and rice (Adinde *et al.*, 2020) under drought stress and controlled condition (Abiri *et al.*, 2016). Our results in parallel to those obtained by Elkoca (2014), who found that osmo- priming of *Pisum sativum* L. cv. Winner seeds with -0.5 bar by PEG and mannitol, as well as hydropriming led to an increase in the germination rate. Pirasteh-Anosheh and Hashemi (2020) declare the positive effect of seed priming on germination, emergence, growth, yield and biochemical content under saline and non-saline condition. Afrin (2021) reported that the highest germination percentage of *Triticum aestivum* L. seeds was 98% and 98.33% due to osmo- priming with 2% mannitol and 5% PEG solution for 9 hours. While, the lowest germination rate (90.83%) was obtained for primed seeds placed in 20% PEG.

Water deficit condition inhibits cell division and elongation that leading to shoot length reduction and a sort of tuberization and lignification of the root. Thus, the slowdown in its growth, and waiting for a favorable condition to grow (Taiz & Zeiger, 2006). The data belongs to the growth characters of the seedlings were taken after 12 days from germination organized in Table 2. I It seems that the first method of priming (P1) by soaking the seeds for 24 hours in sterilized distilled water and later on subjected to the first level of drought stress (G1P0D1) affected significantly on the plumule length to be the highest value (7.33 cm) recorded as compared to other treatment combinations, but being not significantly differ from control condition without inducing drought stress (G1P0D0). While the radicle length was differed significantly in the drought stressed treatment former its priming in water (G1P0D1) to have 3.98 cm. As well as, soaking the seeds in the 100 g/L (P2) and later exposure to -0.79 bars osmotic potential (G1P2D1). Similarly, G1P2D1 treatment led to a significant induce of radicle elongation (4.00 cm), to declare the effective role of priming with high osmotic potential to warn the plant before the stress to enhance the elongation of radicle under drought condition and even under control condition (G1P2D0) to had 4.12 cm radicle length. The results in accordance with those obtained by Afrin (2021) revealed that the under-drought stress highest shoot and root length recorded when the seeds were primed with 5% PEG. Whereas the lowest shoot and root length was observed from primed seeds in 20% PEG. Li *et al.* (2020) reported that 300 μmol L^{-1} of melatonin copped the negative effect of drought stress and increased radicle length, radicle number, and plumule and root length of the seedlings. The plumule and radicle dry weights (PDW and RDW) exhibited a highest significant increase due to G1P0D1 and G1P2D1 (0.035 and 0.031) and (0.037 and 0.030). It means the tolerance of Se-mahee seedlings (G1) increased to the first level of drought stress (D1) due to its previous priming in water (P1) and 100 g/L PEG-8000 (P2). The lowest values of PL, PDW, and RDW were (1.14 cm, 0.014 cm, and 0.013 cm) and (1.12 cm, 0.012 g, and 0.011 g) respectively recorded by G2P0D2 and G2P2D2, while the lowest values of RL and R: P were recorded by G2P1D1 2.00 cm and 0.43. The results obtained by Afrin (2021) showed that the highest shoot and root dry weight was observed in primed seeds placed with 5% PEG solution where the lowest was observed in primed seeds placed with 20% PEG solution. While, Hameed and Iqbal (2013) found that the mannose primer solution increased the dry biomass of wheat shoots and roots under water scarcity conditions. Thiruppathi *et al.* (2018) reported that 2% $ZnSO_4$ as the most promising priming solution for enhancing seedling growth and its tolerance to drought. Oppositely the R: P of Shesh-mahee (G2) was increased significantly and was superior to Se-mahee. The highest ratio was recorded by G2P0D2 and G2P2D2, which means that the seedling tried to cope with the stress by increasing the biomass allocation to the radicle, which might be related to its previous hardening in water (P0) and 100 g/L (P2). Plants allocate more food to the roots than shoots, which leads to an increase in root biomass under water shortage condition

Table 2: Effect of drought stress levels and priming types on the seedling growth characters of two Kurdish rice genotypes

	Plumule length (cm)	Radicle length (cm)	Plumule dry weight (g)	Radicle dry weight (g)	Radicle to plumule ratio (R: P)
G1P0D0	7.01±0.17^{a}	3.34±0.31^{ab}	0.034±0.002^{ab}	0.028±0.001^{ab}	0.87±0.07^{bc}
G1P0D1	7.33±0.67^{a}	3.98±0.01^{a}	0.035±0.002^{a}	0.031±0.005^{a}	0.54±0.03^{cd}
G1P0D2	3.63±0.21^{de}	3.21±0.34^{ab}	0.021±0.001^{d}	0.026±0.001^{ab}	0.87±0.05^{bcd}
G1P1D0	3.50±0.29^{de}	3.66±0.42^{ab}	0.021±0.001^{d}	0.021±0.003^{de}	1.05±0.05^{bcd}
G1P1D1	6.83±0.16^{ab}	3.67±0.21^{ab}	0.033±0.001^{ab}	0.023±0.005^{cde}	0.53±0.03^{cd}
G1P1D2	5.00±0.58^{cd}	3.31±0.11^{ab}	0.022±0.002^{d}	0.024±0.001^{cd}	0.67±0.04^{bcd}
G1P2D0	3.83±0.15^{de}	4.12±0.17^{a}	0.024±0.001^{cd}	0.023±0.002^{cde}	1.09±0.04^{bc}
G1P2D1	3.83±0.17^{cd}	4.00±0.01^{a}	0.037±0.008^{a}	0.030±0.004^{a}	0.56±0.05^{cd}
G1P2D2	3.83±0.61^{de}	3.33±0.34^{ab}	0.023±0.001^{d}	0.027±0.001^{ab}	0.89±0.06^{bcd}
G2P0D0	3.67±0.34^{de}	3.34±0.41^{ab}	0.028±0.005^{bc}	0.022±0.001^{e}	0.92±0.08^{bcd}
G2P0D1	2.35±0.24^{ef}	2.31±0.11^{bc}	0.020±0.004^{d}	0.015±0.003^{f}	1.01±0.01^{bcd}
G2P0D2	1.14±0.12^{f}	3.13±0.13^{ab}	0.014±0.001^{e}	0.013±0.03^{g}	2.80±0.25^{a}
G2P1D0	2.33±0.32^{ef}	2.17±0.17^{bc}	0.023±0.001^{d}	0.021±0.03^{de}	0.97±0.17^{bcd}
G2P1D1	4.83±0.61^{cd}	2.00±0.03^{c}	0.024±0.004^{cd}	0.027±0.004^{ab}	0.43±0.05^{d}
G2P1D2	2.67±0.34^{ef}	3.17±0.17^{ab}	0.018±0.007^{d}	0.016±0.005^{f}	1.22±0.15^{b}
G2P2D0	5.33±0.19^{bc}	3.00±0.29^{abc}	0.020±0.001^{d}	0.020±0.005^{e}	0.57±0.07^{cd}
G2P2D1	2.33±0.34^{ef}	2.33±0.31^{bc}	0.018±0.003^{d}	0.015±0.005^{f}	1.00±0.01^{bcd}
G2P2D2	1.12±0.17^{f}	2.12±0.17^{ab}	0.012±0.001^{e}	0.011±0.04^{g}	2.83±0.45^{a}

(Mahmood *et al*., 2024). A greater root system ensures a greater water uptake under drought condition which promise the shoot system to keep hydrated and maintain a regular growth under stress conditions (dos Santos *et al*., 2022). Many plants respond to drought by developing deeper roots and thus, increase the root: shoot ratio as previously reported by Ahmad *et al.* (2017), Qadir (2018), Othmani *et al.* (2021) and Qadir *et al.* (2022).

The data represented in Figure 1 represents the seedling vigor index (SVI) for different treatments related to the priming methods and drought stress levels of Se-mahee (G1) and Shesh-mahee (G2). SVI can be regarded as an index to evaluate the degree of tolerance to drought stress of the genotype (Liu *et al*., 2015). The highest values of the index were 10.01, 8.75, and 9.10 reported by Se-mahee (G1) when germinated under control (D0) and moderate drought stress (D1) with previous hydropriming, and 5% PEG solution (G1P0D0, G1P0D, and G1P1D1 respectively) with no significant difference among them. It means that G1 could tolerate the stress at a higher rate. The lowest values of SVI were 1.21, 1.94, and 0.97 recorded by G2P0D2, G2P1D2, and G2P2D2 respectively where they were statistically similar in their vigor, which means the sensitivity of G2 to drought stress even with previous priming methods. The shoot and root length reduction reported under drought condition (Table 2) and the reduction in germination rate (Table 1) affected the vigor of the seedling. Because the seedling growth depends on the ability of cells to divide, elongate and differentiate (Taiz & Zeiger, 2006). While priming the seeds before drought stress inducement helps the seeds to activate necessary enzymes for embryo sustainability and make a signal for Giberillic acid synthesis to start the germination process before the stress (Marthandan *et al*., 2020). The results in parallel with those reported by Uddin *et al.* (2021) that the vigor index decreased with the increase in PEG concentration which evidenced the negative effect of drought on plumule and radicle elongation. It was reported previously by Charachimwe *et al.* (2023) that, Osmo priming using PEG 20% given a higher germination rate, shoot and root length, fresh and dry weight of wheat seedling vigor index. As well as 1% mannitol priming given the highest vigor index and seedling length of broad bean (Cokkizgin *et al*., 2019). Qadir (2021) showed the significant effect of priming in increasing the radicle and plumule length and seedling vigor in different bread wheat genotypes.

Stress index (SI) of both plumule and radicle length (PLSI and RLSI) determined the tolerance of rice genotypes to the stress condition. A genotype with a higher SI value has a higher ability to perform better sustainability under stress conditions. Previous priming of the seeds might play a significant role over all germination process (Qadir, 2021), in which may enable the seedling to withstand the stress later. Both PLSI and RLSI were significantly affected by the priming methods under control and drought conditions in the two rice genotypes (Figure 2). The plumule and radicle length stress index (PLSI and RLSI) were maximum in G1P0D2 and G1P2D1 (1.45 and 1.21) and (1.43 and 1.20). In contrast, the minimum PLSI were G2P0D2 0.31 and 0.32 recorded by G2P2D2, but the minimum RLSI were 0.60 and 0.61 recorded by G2P1D2 and G2P1D1. Which remark the Se-mahee genotype as more tolerant than Shesh-mahee under moderate and severe drought condition. The shoot growth continued but at a slow rate under mild sometimes severe drought compared to water available condition. Stomata will be closed and CO_2 uptake decreased that a lower rate of photosynthesis and assimilation which reduces the amount of cellular energy (ATP) for cell division and differentiation and the cells lose their turgidity. As well as a decline in gibberellin levels reduce the plumule growth (Taiz & Zeiger, 2006). But it seems that hydropriming and osmo- priming (10% PEG) acted as a previous alarm for G1 to harden the stress before its exposure and could elongate their shoot and continue their growth. The root length of some plants will be increased to reach available water in deeper zones, which means that the plant avoids the stress and try to complete its life cycle faster (Farooq *et al*., 2024).

The plumule and radicle elongation speed (PES and RES) of rice genotypes were significantly affected by the drought stress treatments and priming solutions (Figure 3). The highest elongation speed of both plumule and radicle was 0.63 and 0.33, and 0.61 and 0.33 reported via treatment combinations G1P0D1 and G1P2D1. That means the effectiveness of hydropriming (P0) and 10% PEG (P2) on the elongation process of Se-mahee genotype under drought conditions. While the lowest value of

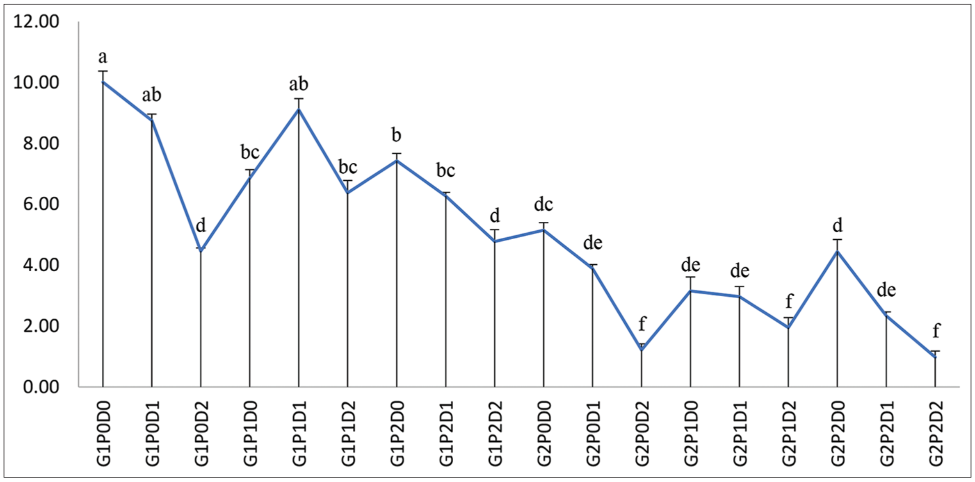

Figure 1: Effect of drought stress levels and priming method on the seedling vigor index (SVI) of two Kurdish rice genotypes

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PES was 0.0972 as a response of Shesh-mahee (G2) to drought stress G2P0D2 and G2P2D2 that means the genotype couldn't tolerate the severe drought stress despite its previous priming methods used. As well as the lowest RES was 0.17 recorded by G2P1D1, Shesh-mahee under all combined treatments of priming and drought stress showed their sensitivity to water shortage that they couldn't combat the stress and elongate their root under such conditions. Violita and Azhari (2021) found that the water uptake decreased by the seeds as the water shortage increased which led to disturbance in cell metabolism and cell division and elongation. Increased root elongation in shorter times (RES) is an attempt of the plant to find water as soon as possible to escape the stress and shorting its life cycle, that regarded as an indicator of rice being tolerant to water shortages as previously found by Hussain *et al.* (2018), Zagoto and Violita (2019), Violita and Azhari (2021) and Siddique *et al.* (2023).

Relative water content (LRW) could be regarded as an important sign of tolerant crop plants to drought stress (Qadir *et al.*, 2022). The RWC of the two Kurdish rice genotypes showed statistically significant variation due to the priming methods of the seeds and drought stress levels (Figure 4). The water content of the seedlings decreased due to a decrease in available water. The highest values were 0.24 and recorded by G1P2D1 and G1P1D1, means that the osmo-priming of the seeds had a significant effect in decreasing the effect of moderate drought stress of Se-mahee genotype, while Shesh-mahee genotype couldn't cope with the stress even though their previous priming in which the lowest RWC were 0.10 and 0.11 recorded by G2P2D2 and G2P0D2. The results in accordance with Faijunnahar *et al.* (2017) and Azmat *et al.* (2020) reported that seed priming led to an increase in germination rate, growth characters of the seedlings and water content and behaviors of wheat genotypes. As well as in parallel with Afrin (2021) who found that the highest relative water content was recorded due to hydropriming and 5% PEG under drought stress conditions and the lowest relative water content was due to 20% PEG solution.

Water saturation deficit (WSD) was varied significantly between the two genotypes under the effect of drought stress and the priming methods used (Figure 5). The results showed that the water saturation deficit increased significantly with increasing PEG levels in Shesh-mahee genotype compared to Se-mahee. Results revealed that the highest values of WSD were 0.90, 0.88 and 0.89 due to G2P0D2, G2P2D1, and G2P2D2. The lowest values were 0.76 and 0.75 recorded by G1P1D1 and G1P2D1. Se-mahee genotype could tolerate the low of drought stress due to osmo- priming at the both

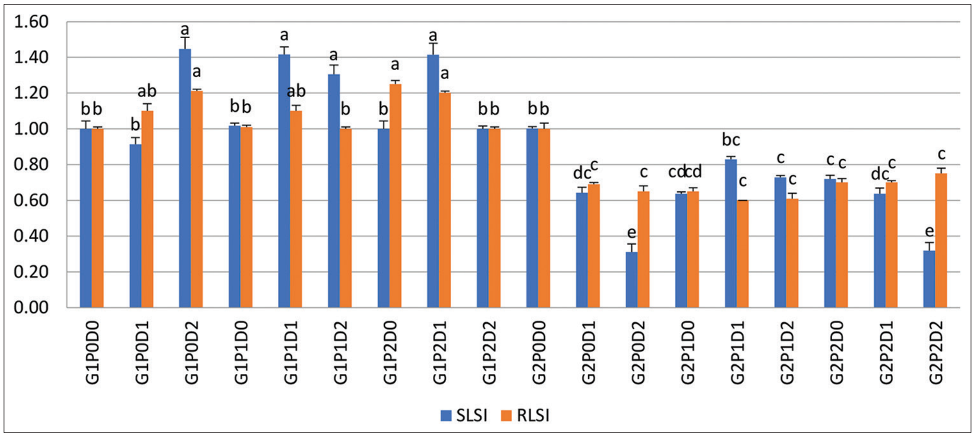

Figure 2: Effect of drought stress levels and priming methods on plumule and radicle length stress index (PLSI and RLSI) of two Kurdish rice genotypes

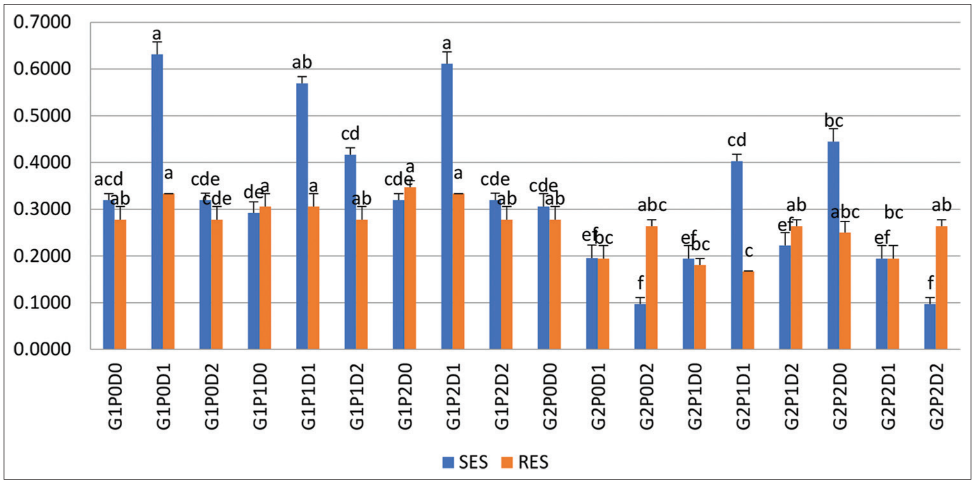

Figure 3: Effect of drought stress levels and priming methods on plumule and radicle elongation speed (RES and SES) of two Kurdish rice genotypes

levels 5 and 10 PEG g/L, this result is in parallel with Faijunnahar *et al.* (2017), they found that osmo- priming might have a role in recovering the physiological damages under drought condition and minimize the water saturation deficit over priming time that accelerate the ageing process and produced weak and sensitive seedlings couldn't uptake enough water for imbibition and seed germination, means that the available water for saturation will be reduced (Afrin, 2021). But Shesh-mahee couldn't tolerate the drought stress despite to the pre priming methods used, which is in agreement with Baque *et al.* (2018) who claimed that the water saturation deficit of primed wheat genotypes was gradually increased with increasing stress condition.

A significant variation was observed in proline content in response to drought stress and previous priming solutions (Figure 6). The highest content of proline detected from

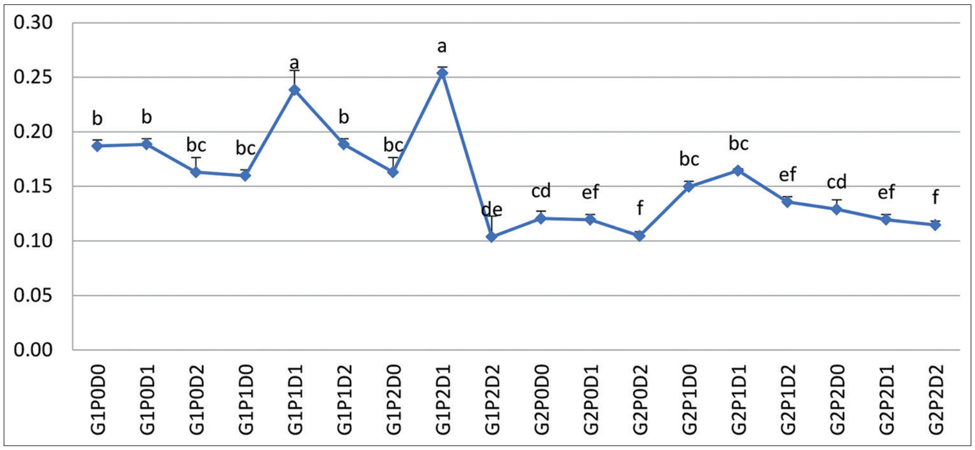

Figure 4: Effect of drought stress levels and priming methods on relative water content (RWC) of two Kurdish rice genotype seedlings

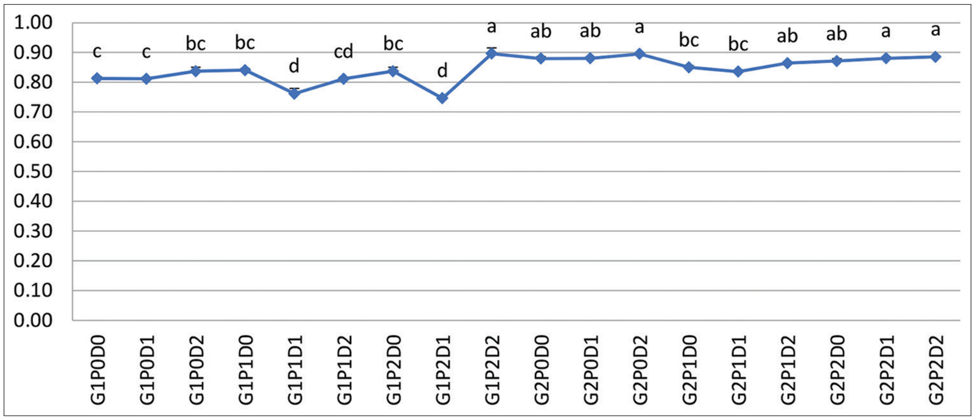

Figure 5: Effect of drought stress levels and priming methods on water saturation deficit (WSD) of two Kurdish rice genotype seedlings

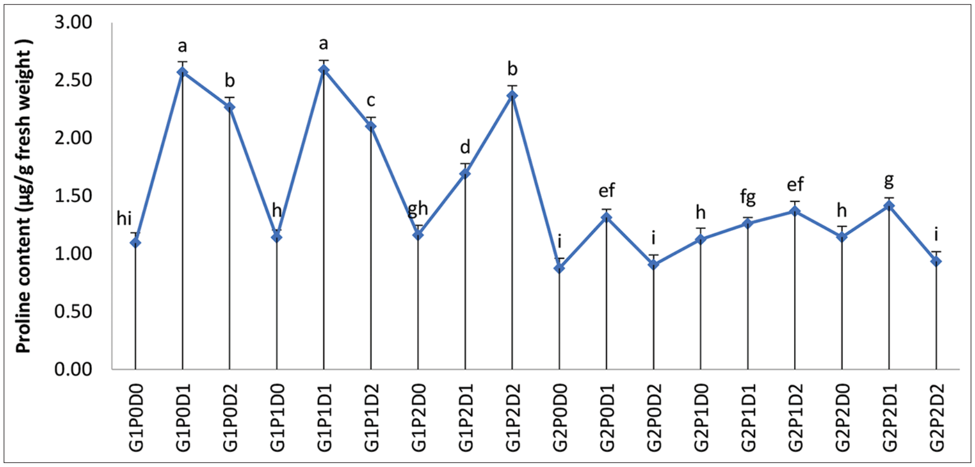

Figure 6: Effect of drought stress levels and priming methods on proline content (µg/g fresh weight) of two Kurdish rice genotype seedlings

Se-mahee genotype (G1) under the first level of drought stress (D1) and its hydro priming (P0) and osmo- priming (P1) were 2.57 and 2.59 μg/g fresh weight due to G1P0D1 and G1P1D1. That means the tolerance of G1 to drought stress, because proline accumulation in plants has been identified to be an indicator of the plant tolerance to drought stress (Barunawati *et al.*, 2016). While Shesh-mahee genotype couldn't tolerate the drought stress levels and the priming methods didn't affect significantly in decreasing the sensitivity of the genotype to the water shortage, in which the lowest values of proline were 0.90 and 0.93 μg/g fresh weight recorded by G2P0D2 and G2P2D2, as previously proven by Purbajanti *et al.* (2017), Chaniago *et al.* (2021) and Hartatik *et al.* (2024) in rice plants and Barunawati *et al.* (2016), Qadir (2018) and Qadir *et al.* (2022) in wheat plants. Proline is an amino plays an important role as an osmo-regulator and keeps the turgor pressure of the cell to enhance root growth to deeper zones of the soil during periods of drought (Zivcak *et al.*, 2016).

CONCLUSION

Hydropriming and 5% PEG significantly decrease the sensitivity of Se-mahee rice under water scarcity by enhancing seed germination, seedling growth, and vigor, while Shesh-mahee showed a limited response under drought conditions. The study highlights the effectiveness of priming methods as an effective strategy for improving the resilience of rice to drought stress. Due to their role as pre- activator methods for necessary enzymes before the growth under the stress condition which support the seedling growth and performance in water-limited conditions. Finally, PEG can be concluded as an osmo- protectant and osmo- regulator agent during drought conditions.

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